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Metabolic Alterations in End-Stage and Less Severe Heart Failure – Myocardial Carnitine Decrease[1])

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Summary: Severe tissue carnitine deficiency impairs fatty acid oxidation. In explanted hearts from patients with end stage heart failure a 57% carnitine decrease was found in comparison with healthy donor hearts ($p < 0.05$). The reduction of myocardial carnitine levels affected all areas of the explanted hearts to a comparable extent. Carnitine decreases in patients with dilated cardiomyopathy or coronary artery disease were similar.

Endomyocardial biopsies from patients with less severe heart failure due to cardiomyopathy ($n = 28$) or other myocardial diseases ($n = 8$) showed a 42% decrease of total myocardial carnitine (in nmol/mg non-collagen protein) in comparison with biopsies from patients with normal cardiac function (controls) (heart failure: 5.7, confidence interval 4.2–7.0; controls 9.3, confidence interval 7.6–12.0, $p < 0.005$). Free myocardial carnitine in heart failure was also different from controls (heart failure: 4.2, confidence interval 3.7–5.3; controls 10.3, confidence interval 7.5–12.2, $p < 0.001$). The decrease of free and total myocardial carnitine was comparable in dilated cardiomyopathy and heart failure due to other diseases. Alterations in myocardial carnitine content represent therefore non-specific biochemical markers in heart failure with yet unknown consequences for myocardial function.

Introduction

In dilated cardiomyopathy, primary metabolic defects probably cause an impairment of cardiac function. In heart failure due to coronary, valvular or hypertensive heart disease an abnormal haemodynamic burden leads to hypertrophy and secondary metabolic changes (1). Since the lack of adequate amounts of tissue for biochemical studies has always limited the investigation of myocardial metabolism in human heart disease, little information on these metabolic defects is available.

However, as myocardial tissue can now be obtained at cardiac transplantation, metabolic defects can be assessed in end-stage heart failure and the homogeneity and distribution of the changes can be studied. In addition, micromethods as well as adequate reference systems have been developed that enable the investigation of endomyocardial biopsies from patients with less severe stages of heart failure (2). Thus, it is now possible to identify metabolic defects in end-stage heart failure and to search for corresponding changes in endomyocardial biopsies from patients in the early stages of heart failure.

Fatty acid oxidation is the major energy source of the heart (3). Carnitine plays an essential role in mitochondrial fatty acid uptake, and patients with severe carnitine deficiency are almost unable to oxidize lipids (4, 5). They present with severe lipid accumulation in most tissues, with muscle weakness and, often, with

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clinical signs of dilated cardiomyopathy (6, 7). Since there are indicators that myocardial fatty acid oxidation may be disturbed in patients with dilated cardiomyopathy, we decided to study the myocardial carnitine content as a parameter for the capacity to oxidize lipids in patients with heart failure (8).

In the first part of the study, carnitine was measured in explanted hearts to evaluate whether a carnitine loss occurs in heart failure and which areas of the heart are involved. Subsequently, measurements were undertaken on endomyocardial biopsies to determine whether patients who have not yet reached end-stage heart failure have defects in carnitine metabolism.

Methods

Studies in explanted hearts

In a first series of experiments myocardial carnitine content was measured in the explanted hearts of 66 patients who had undergone heart transplantation because of end-stage heart failure. Thirty nine of 66 patients had dilated cardiomyopathy and 27 had coronary artery disease. Both patient groups had a comparable reduction in left ventricular ejection fraction (dilated cardiomyopathy: 21%, coronary artery disease: 22%), cardiac index (dilated cardiomyopathy: 2.1 l/min · m^2, coronary artery disease: 2.3 l/min · m^2) and left ventricular end-diastolic pressure (dilated cardiomyopathy: 22 mmHg, coronary artery disease: 22 mmHg).

Samples for the measurement of total carnitine were taken in all patients from the base of the left ventricle and from the free wall of the right ventricle. In addition, samples from the middle (41/65) and from the apex of the left ventricle (44/65), from the septum interventriculare (35/66), and from the right atrium (46/66) were studied. In 25 patients, samples from 6 areas of the heart (base, middle, and apex of the left ventricle, septum interventriculare, right ventricle, and right atrium) were taken. These samples were used to study the regional distribution of total carnitine in failing hearts. All samples were taken in the operating room, less than 15 min after explantation of the heart, immediately frozen on dry ice and stored at −70 °C until analysis.

Free carnitine was not determined in the explanted hearts because the time between explantation and freezing was between 15 and 30 min. This does not influence total carnitine levels; but carnitine esters may accumulate in this period of global ischaemia and thus, the relative amounts of free and total carnitine will be altered (9).

Left ventricular biopsies from healthy donor hearts (n = 10) and right atrial biopsies obtained at aortocoronary bypass surgery from patients without heart failure (n = 14) were used as controls.

Tab. 1. Haemodynamic parameters in patients with dilated cardiomyopathy (1a), with heart failure due to other causes (1b) and in normal controls (1c).

a) Heart failure due to dilated cardiomyopathy

Patient	Left ventricular end-diastolic pressure (mm Hg)	Right ventricular ejection fraction (%)	Left ventricular ejection fraction (%)	Left ventricular end-diastolic volume index (ml/m^2)	Left ventricular end-systolic volume index (ml/m^2)	Cardiac index ($ml/m^2 \times min$)
1	40	–	31	224	156	2.9
2	12	31	35	212	136	4.5
3	8	41	40	162	97	3.8
4	30	16	12	300	262	4.8
5	18	59	33	279	187	4.1
6	25	28	16	257	216	2.9
7	10	41	39	160	98	3.8
8	9	49	33	156	105	3.8
9	9	50	43	146	84	2.8
10	12	45	44	156	88	2.3
11	21	49	53	180	84	5.6
12	24	43	37	167	105	4.6
13	16	60	44	178	100	3.6
14	23	27	19	328	266	2.1
15	35	34	19	245	199	3.3
16	4	50	33	141	95	2.9
17	20	–	15	178	137	2.1
18	12	20	20	351	200	2.1
19	4	40	45	125	68	3.1
20	22	53	43	153	87	3.4
21	26	28	30	200	139	2.7
22	20	54	30	157	110	3.1
23	30	41	19	140	114	3.6
24	35	15	12	242	212	3.3
25	28	34	26	180	132	2.6
26	6	41	41	131	77	3.9
27	26	29	20	247	197	3.1
28	13	44	34	194	128	3.7
Mean	19	39	31	200	193	3
SE	2	2	2	13	11	0.2

– not determined

b) Heart failure of different origin

Patient	Left ventricular end-diastolic pressure (mm Hg)	Right ventricular ejection fraction (%)	Left ventricular ejection fraction (%)	Left ventricular end-diastolic volume index (ml/m^2)	Left ventricular end-systolic volume index (ml/m^2)	Cardiac index ($ml/m^2 \times min$)
1	8	–	50	140	70	4.2
2	15	39	24	219	167	3.9
3	20	47	31	318	220	4.4
4	8	–	39	158	97	2.2
5	14	55	28	236	107	3.6
6	19	–	25	179	134	3.1
7	18	66	26	303	226	5.0
8	20	55	51	131	64	6.0
Mean	16	52	34	211	136	4
SE	2	2	4	26	22	0.4

– not determined

c) Controls

Patient	Left ventricular end-diastolic pressure (mm Hg)	Right ventricular ejection fraction (%)	Left ventricular ejection fraction (%)	Left ventricular end-diastolic volume index (ml/m^2)	Left ventricular end-systolic volume index (ml/m^2)	Cardiac index (ml/m^2)
1	11	49	64	156	57	4.0
2	17	54	67	111	37	2.8
3	9	60	67	112	36	3.9
4	24	62	59	126	52	5.6
5	10	–	62	75	28	3.1
6	10	–	67	129	42	2.3
7	11	56	73	85	23	3.5
8	20	–	63	109	41	5.1
9	9	56	63	90	33	5.4
10	7	60	69	83	25	3.6
11	12	58	72	131	36	3.8
12	6	–	57	160	69	4.8
13	13	57	64	107	39	5.0
Mean	12	58	65	111	39	4
SE	1	2	1	9	3	0.3

– not determined

Studies in endomyocardial biopsies

A second part of the study comprised 28 adults with dilated cardiomyopathy, 8 with heart failure of other origins, and 13 controls without heart disease. After an overnight fast, all subjects underwent complete non-invasive and invasive diagnostic studies including right and left sided heart catheterization, determination of cardiac index by thermodilution technique, biplane right and left ventricular angiography, coronary angiography, and right ventricular endomyocardial biopsy. The diagnosis of dilated cardiomyopathy was based on a left ventricular ejection fraction of $< 55\%$ after exclusion of primary coronary, valvular, hypertensive, or pulmonary heart disease and myocarditis (tab. 1a). Of the 8 patients whose heart failure was attributed to causes other than dilated cardiomyopathy, 3 had predominantly coronary artery disease, 3 had valvular, and 2 had hypertensive heart disease (tab. 1b).

The 13 controls were subjects in whom invasive diagnostic examination was undertaken for suspected coronary artery disease and in whom normal coronary arteries together with a normal left ventricular ejection fraction ($> 55\%$), without valvular, hypertensive or pulmonary heart disease, were found (tab. 1c).

Total myocardial carnitine was measured in biopsies from all patients and controls. Biopsies were frozen on dry ice less than 20 s after sampling. This short interval should not allow significant accumulation of carnitine esters. Therefore, if possible (depending on sample size), free myocardial carnitine was determined in the same biopsies (22 patients and 11 controls) and the ratio free/total carnitine was calculated separatedly for every sample.

Biochemical determinations

Myocardial carnitine was measured using a radio-enzymatic standard assay and microassay, specially adapted for endomyocardial biopsy, as published recently (2, 10). Total carnitine was measured directly after alkaline hydrolysis of plasma or tissue homogenate. Free carnitine was determined after acid extraction and neutralization of the sample (9).

Non-collagen protein was used as a reference system. Collagen protein was first extracted from the diluted tissue homogenate (11), followed by a conventional protein determination (12).

Based on our previous studies, the intra-assay coefficients of variation for total and free carnitine in concentrations from 50 to 500 pmol are 4% and 9%, respectively. The recovery rate for externally added free and acetyl-carnitine in amounts from 25 to 100 nmol/sample are $95 \pm 8\%$ (free) and $92 \pm 14\%$ (acetyl-carnitine). The coefficient of variance for determinations from several biopsy-sized samples from the same heart is 13%, with a 95% confidence interval of 1.9 nmol carnitine per milligram non-collagen protein (2). Micro- and regular assay yield identical results.

Statistics

Data are given as median and confidence interval. The U-test of *Wilcoxon, Mann & Whitney* was used to compare groups, and values of $p < 0.05$ were considered significant.

To compare the carnitine content of different areas of the explanted hearts, independent of the absolute levels, samples were ranked from lowest (1) to highest (6) carnitine content in each of the 25 patients where carnitine levels were determined in 6 areas. The average rank for the samples at each location was then calculated for each area.

For the patients that were not yet in end-stage heart failure, regression analysis was done between free myocardial carnitine (x, = independent variable) and left ventricular ejection fraction, (y, = dependent variable) using $y = a + b/x$ as a non-linear curve fitting model.

Results

Myocardial carnitine content in end-stage heart failure — Measurements in explanted hearts

Sixty six patients with heart failure due to dilated cardiomyopathy or coronary artery disease had significantly reduced myocardial carnitine concentrations in all areas of the explanted hearts, compared with control values. Carnitine levels (in nmol/mg non-collagen protein) in patients were: left ventricular base 5.7, left ventricular mid 6.2, left ventricular apex 5.8, septum 5.3, right ventricle 5.8, right atrium 4.8. Control values were 13.3, confidence interval 9.7 – 19.4 in the left ventricle and 11.7, confidence interval 9.8 – 14.1 in the right atrium ($p < 0.05$ for all areas). Patients with dilated cardiomyopathy and coronary artery disease showed no differences in myocardial carnitine content. In both groups the decrease of carnitine affected all areas of the heart (fig. 1).

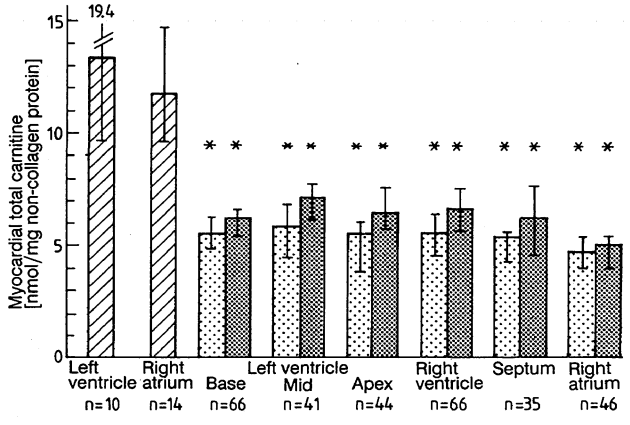

Fig. 1. Myocardial total carnitine content in patients with heart failure disease and in controls. Median and confidence interval.
▨ Controls
⊞ Dilated cardiomyopathy
▩ Coronary artery disease
* $p < 0.05$ vs controls (right atrium + left ventricle)

The distribution of carnitine independent of the absolute values was obtained by an analysis by rank (fig. 2). Highest carnitine concentrations were found at the base of the left ventricle, followed by the mid area of the left ventricle with comparable levels in right ventricle, septum, and left ventricular apex. The lowest carnitine content was found in the right atrium (fig. 2).

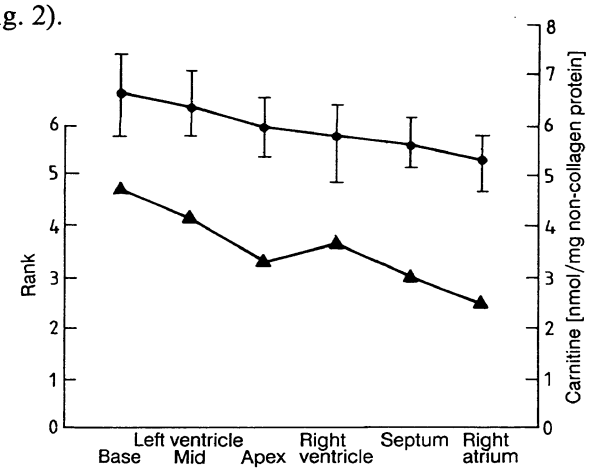

Fig. 2. Average ranks for the carnitine concentrations in each of 6 areas of the heart (triangles) and absolute carnitine concentrations (circles) in the corresponding areas.
To compare the carnitine content in different areas of the explanted hearts, independent of the absolute levels, samples were ranked from lowest (1) to highest (6) carnitine content in each of the 25 patients; carnitine levels were determined in 6 areas. The average rank for the samples at each location was then calculated for each area.

Myocardial carnitine content in less severe stages of heart failure — Measurements in endomyocardial biopsies

Patients with heart failure showed a significant decrease of myocardial carnitine (in nmol/mg non-collagen protein) compared with controls (heart failure: 5.7, confidence interval 4.2 – 7.0; controls: 10.3, confidence interval 7.6 – 12.0, $p < 0.005$). There was a tendency towards a greater myocardial carnitine decrease in patients with left ventricular ejection fractions < 30% (52% carnitine loss) than in patients with left ventricular ejection fractions 30 – 55% (39% carnitine loss) (fig. 3a). A comparable decrease of total myocardial carnitine was found in patients with heart failure caused by dilated cardiomyopathy and patients with heart failure of different origins (fig. 3b).

Free myocardial carnitine (in nmol/mg non-collagen protein) in 22 patients with heart failure was significantly reduced compared with 11 normal controls (heart failure: 4.2, confidence interval 3.7 – 5.3, controls: 10.3, confidence interval 7.5 – 12.2, $p < 0.001$). Differences between patients with left ventricular ejection fraction < 30% and left ventricular ejection frac-

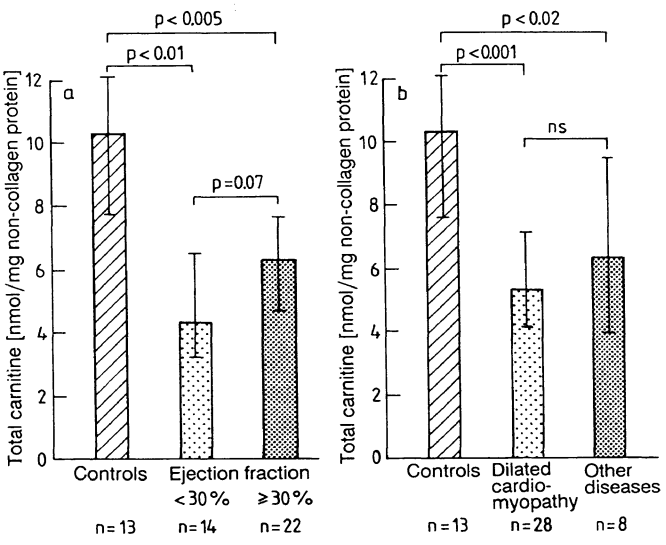

Fig. 3. Myocardial total carnitine content in
a) patients with congestive heart failure and left ventricular ejection fraction < 30% or 30–55% and controls. Median and confidence interval.
b) patients with dilated cardiomyopathy, with congestive heart failure due to other diseases and controls. Median and confidence interval.

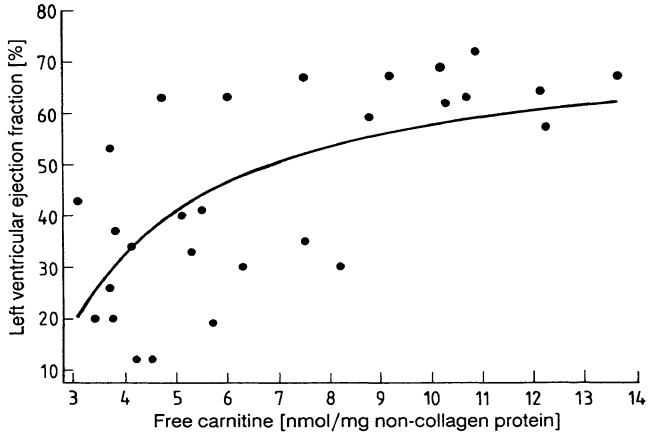

Fig. 5. Results of non-linear regression analysis between free myocardial carnitine content and left ventricular ejection fraction demonstrating a non-linear correlation. If the equation of the curve fitting model is transformed with Z = 1/free carnitine as the new independent variable, a linear correlation coefficient of r = 0.67 is obtained.

Left ventricular ejection fraction =

$$74.58 - \frac{167.29}{\text{free carnitine}}$$

tion 30–55% were not significant (fig. 4a). Free myocardial carnitine did not differ between patients with heart failure due to dilated cardiomyopathy or to coronary or valvular disease (fig. 4b). A non-linear relation was found between free myocardial carnitine and left ventricular ejection fraction (fig. 5).

The ratio, myocardial free/total carnitine, was 0.81 ± 0.05 in the heart failure group and 0.91 ± 0.03 in the control group; the difference was not significant.

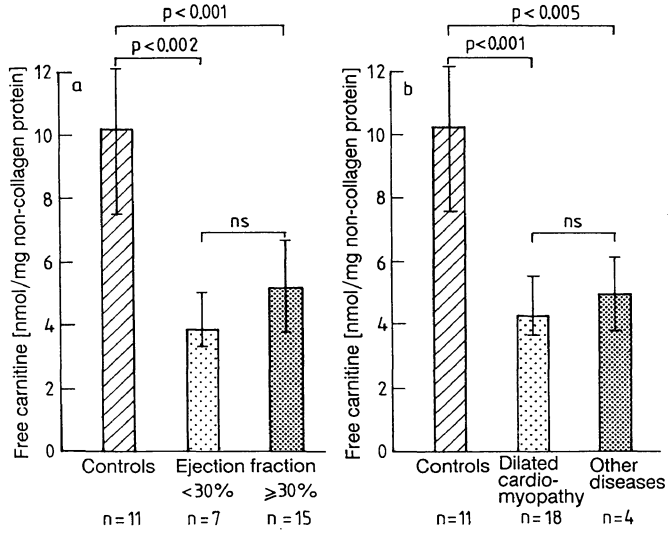

Fig. 4. Myocardial free carnitine content in
a) patients with congestive heart failure and left ventricular ejection fraction < 30% or 30–55% and controls. Median and confidence interval.
b) patients with dilated cardiomyopathy, with heart failure due to other diseases and controls. Median and confidence interval.

Discussion

Carnitine, a low molecular weight amino acid derivative, is essential for the oxidation of fatty acids, the preferred source of energy for the heart (3, 13). Severe tissue carnitine depletion impairs fatty acid oxidation and thus leads to cardiac failure (4–7). We found a significant myocardial carnitine decrease in explanted hearts with end stage heart failure and confirmed a similar decrease in endomyocardial biopsies from patients with less severe disease. Studying the same metabolic alterations first in explanted hearts and later in endomyocardial biopsies offers several advantages: the range between normal and end stage disease as well as the reproducibility of measurements and regional distribution of metabolites can be determined on large samples and serves as a basis for the investigation of biopsies.

In the explanted hearts, no differences were found between carnitine levels in the left ventricle and right ventricular septum. We and others found significant correlations between biochemical changes in the right ventricular septum and functional parameters in the left ventricle (14, 15). Thus, the septum probably resembles in its biochemical properties the left ventricle, and biopsies from the right ventricular septum may be used to estimate the carnitine content in the left ventricle.

The use of an appropriate reference system is of major importance for obtaining accurate metabolite levels in endomyocardial biopsies. The variability between

different samples from the same area of the heart is much more pronounced when wet weight rather than non-collagen protein is used as a reference system. Since this variability increases with decreasing sample size it appears to be optimal to relate metabolite levels in biopsies to non-collagen protein or to other more specific reference systems (2).

Patients with heart failure due to dilated cardiomyopathy or to other cardiac diseases revealed a comparable reduction in myocardial carnitine. Thus, alterations in carnitine metabolism apparently represent metabolic changes associated with heart failure, regardless of its origin (16, 17).

A decrease of myocardial carnitine is not only present in end stage heart failure but is also found in less severe disease. Free myocardial carnitine represents the important part of the carnitine pool that can actively participate in the transesterification of cytosolic fatty acid coenzyme A esters. In our patients, relatively high ratios of free/total carnitine were found, possible due to the fact that all patients were studied in the fasting state. The correlation between free myocardial carnitine and the left ventricular ejection fraction suggests an association between the reduction in myocardial carnitine levels and the impairment of cardiac function, in spite of a great interindividual variability. Either impaired accumulation of carnitine may reflect the severity of myocardial damage, or the myocardial carnitine decrease contributes to the reduction of myocardial function – in addition to a considerable heterogeneity of myocardial metabolism in heart failure (2, 16).

Primary as well as secondary carnitine deficiency syndromes have been described (18–20). The patients affected are mainly characterized by low plasma carnitine levels. In contrast, heart failure patients usually present with high plasma carnitine (15, 17). Reduced myocardial carnitine in the presence of high plasma levels may be caused by defects in the synthesis of the carnitine precursor, butyro-betaine, or defects in the carrier system, as in diphtheria (21, 22). In addition, non-specific membrane damage, like that in ischaemia, may be involved in the pathogenesis of carnitine deficiency in heart failure (9).

A possible effect of carnitine supplementation in heart failure depends on myocardial carnitine levels. Thus, proper selection of patients, based on tissue carnitine levels, is required for any therapeutic trials.

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References

1. Katz, A. M. (1989) Changing strategies in the management of heart failure. J. Am. Coll. Cardiol. *13*, 513–523.
2. Regitz, V., Sasse, S., Schüler, S., Yankah, A. C., Hetzer, R. & Fleck, E. (1989) Variabilität von Metabolitkonzentrationen und Enzymaktivitäten im menschlichen Myokard. Z. Herz-, Thorax-, Gefäßchir. *3*, 107–114.
3. Neely, J. R. & Morgan, H. E. (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann. Rev. Physiol. *36*, 413–459.
4. Engel, A. G. & Angelini, C. (1973) Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: a new syndrome. Science *179*, 899–902.
5. Chapoy, P. R., Angelini, C., Brown, W. J., Stiff, J. E., Shug, A. L. & Cederbaum, S. D. (1980) Systemic carnitine deficiency – a treatable inherited lipid storage disease presenting as Reye's syndrome. New England J. Med. *303*, 1389–1393.
6. Waber, L. J., Valle, D., Neill, C., DiMauro, S. & Shug, A. (1982) Carnitine deficiency presenting as familial cardiomyopathy. A treatable defect in carnitine transport. J. Pediat. *101*, 700–708.
7. Regitz, V., Hodach, R. J. & Shug, A. L. (1982) Carnitin-Mangel: eine behandelbare Ursache kindlicher Kardiomyopathien. Klin. Wochenschr. *60*, 393–399.
8. Geltman, E. M., Smith, J. L., Beecher, D., Ludbrook, P., Ter-Pogossian, M. M. & Sobel, B. E. (1983) Altered regional myocardial metabolism in congestive cardiomyopathy detected by positron tomography. Am. J. Med. *74*, 773–785.
9. Shug, A. L., Thomsen, J. H., Folts, J. D., Bittar, N., Klein, M. I., Koke, J. R. & Huth, P. J. (1978) Changes in tissue levels on carnitine and other metabolites during myocardial ischemia and anoxia. Arch. Biochem. Biophys. *187*, 25–33.
10. Parvin, R. & Pande, S. V. (1977) Microdetermination of (–) carnitine and carnitine acetyltransferase activity. Anal. Biochem. *79*, 190–201.
11. Lilienthal, J. L. jr., Zierlier, K. L., Volk, B. P., Bucher, R. & Riley, M. (1950) A reference base for analysis of muscle constituents. J. Biol. Chem. *182*, 501–508.
12. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. Anal. Biochem. *72*, 248–254.
13. Bremer, J. (1983) Carnitine – metabolism and functions. Physiol. Rev. *63*, 1420–1442.
14. Peters, T. J., Wells, G., Oakley, C. M., Brooksby, I. A. B., Jenkins, S. B., Webb-Peploe, M. M: & Coltart, D. J. (1977) Enzymic analysis of endomyocardial biopsy specimens from patients with cardiomyopathies. British Heart Journal *39*, 1333–1339.
15. Unverferth, D. V., Lee, S. W. & Wallick, E. T. (1988) Human myocardial adenosine triphosphatase activities in health and heart failure. Am. Heart. J. *115*, 139–146.
16. Regitz, V., Shug, A. L., Schüler, S., Yankah, A. C. & Hetzer, R. (1988) Herzinsuffizienz bei dilatativer Kardiomyopathie und koronarer Herzerkrankung – Beitrag biochemischer Parameter zur Beurteilung der Prognose. Dtsch. Med. Wochenschr. *113*, 781–786.

17. Regitz, V., Müller, M., Schüler, S., Yankah, C. A., Hetzer, R., Shug, A. L. & Fleck, E. (1987) Carnitinstoffwechsel-Veränderungen im Endstadium der dilatativen Kardiomyopathie und der ischämischen Herzmuskelerkrankung. Zeitschr. Kardiologie *76*, Suppl. *5*, 1–8.
18. Treem, W. R., Stanley, C. A., Finegold, D. N., Hale, D. E. & Coates, P. M. (1988) Primiary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. New England J. Med. *319*, 1331–1336.
19. Roe, C. R., Millington, S. D., Maltby, D. A., Kahler, S. G. & Bohan, T. P. (1984) L-Carnitine therapy in isovaleric acidemia. J. Clin. Invest. *74*, 2290–2295.
20. Winter, S. C., Szabo-Aczel, S., Curry, C. J. R., Hutchinson, H. T., Hogue, R. & Shug, A. L. (1987) Plasma carnitine deficiency: clinical observations in 51 pediatric patients. Am. J. Dis. Child. *141*, 660–665.
21. Siliprandi, N., Di Lisa, F., Pivetta, A., Miotto, G. & Siliprandi, D. (1987) Transport and function of carnitine and propionyl carnitine: relevance to some cardiomyopathies and cardiac ischemia in particular. Zeitschr. Kardiol. *76*, Suppl. *5*, 34–40.
22. Bressler, R. & Wittels, B. (1965) The effect of diphtheria toxin on carnitine metabolism in the heart. Biochim. Biophys. Acta *104*, 39–45.

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